Phytochemistry, 1964, Vol. 3, pp. 229 to 234. Pergamon Press Ltd. Printed in England

# TARAXIEN, THE CAROTENOID ESTER IN DANDELION FLOWERS

V. H. BOOTH*

Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council

(*Received* 11 *September* 1963)

**Abstract**—The principal pigment found in extracts from flowers of *Taraxacum officinale* was a di-ester of taraxanthin for which the name taraxien is suggested. The concentration in the flowers expressed as carotenol, was about 350 ppm fresh wt. Partial saponification produced a mono-ester that could be further saponified to free carotenol. The mono-ester had solubilities and adsorption affinities between those of taraxien and taraxanthin. Appreciable amounts of the mono-ester and of free taraxanthin were found in the flowers. Di-ester, mono-ester and free carotenol each had spectral absorption maxima at 420, 442 and 471 mμ (wavenumbers 23,800, 22,610 and 21,250 c/cm) in both *n*-hexane and ethanol. The properties of free taraxanthin are compared with those of violaxanthin.

KUHN AND LEDERER[1] first isolated taraxanthin as principal pigment from a saponified extract of the flowers of the dandelion, *Taraxacum officinale*. The chemical constitution of this carotenoid is unknown, but Karrer and Jucker[2] pointed out that its molecular formula $C_{40}H_{56}O_4$ shows that the pigment is isomeric with violaxanthin. Karrer and Rutschmann[3] could not find the pigment, and suggested that its occurrence depended on geographical factors. Later, Strain[4] isolated taraxanthin from dandelions and described some of its properties.

The greater part of the pigment in the flower of the dandelion appears to be a di-ester of taraxanthin, with lesser amounts of a mono-ester and of free taraxanthin. The present paper is concerned with these esters.

## RESULTS

### *The Principal Pigments*

The major pigments seen on two-dimensional chromatograms of light-petroleum extracts of the flowers are shown in Fig. 1. Pigment *d*2 was not completely separated from *d*1 at the first chromatographic run. It appears to be a *cis* isomer of *d*1 and may have been produced during manipulations. Pigments *m*2 and *t*2 were similarly presumed to be *cis* isomers of *m*1 and *t*1 respectively. Pigments *d*1 and *d*2 were better separated when 0·5% acetone in light petroleum was used in the first dimension, whereas *t*1 and *t*2 were better separated when 20% acetone was used.

### *Di-ester*

The pigment present in greatest amount, namely *d*1, was eluted into ethanol, and had

* Member of the scientific staff of the Agricultural Research Council.

[1] R. KUHN and E. LEDERER, *Z. physiol. Chem.* **200**, 108 (1931).
[2] P. KARRER and E. JUCKER, *Carotenoids*, translated by E. A. Braude, Elsevier, Amsterdam (1950).
[3] P. KARRER and J. RUTSCHMANN, *Helv. chim. Acta* **25**, 1144 (1942).
[4] H. H. STRAIN, *Arch. Biochem. Biophys.* **48**, 458 (1954).

spectral absorption maxima in both ethanol and *n*-hexane were at 420, 442 and 471 mμ (23,800, 22,610 and 21,250 c/cm). In carbon disulphide, maxima were at 471 and 501 mμ, and in benzene at 429, 453 and 481 mμ.

Pigment *d*1 was epiphasic to light petroleum over aqueous alcohols. On columns of alumina and on paper chromatograms it ran like carotenoid esters, between β-carotene and lycopene. After saponification for 20 min followed by chromatography, most of the pigment was found at position *t*1 with a much smaller amount at *t*2 (Fig. 1). When *d*1 was saponified for only 1 min and chromatographed, all the pigments of Fig. 1 appeared except *c*, the predominant pigment being *m*1 which could be further saponified to produce *t*1. These results suggest that *d*1 is a di-ester.

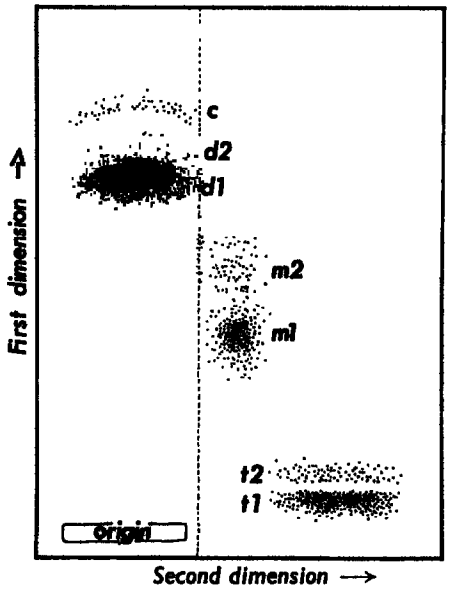


FIG. 1. TWO-DIMENSIONAL PAPER CHROMATOGRAM OF PIGMENTS FROM THE FLOWER OF THE DANDELION (*T. officinale*)

*c*, β-Carotene; $d_1$, $d_2$, *trans* and *cis* di-ester of taraxanthin; $m_1$, $m_2$, *trans* and *cis* mono-ester of taraxanthin; $t_1$, $t_2$, *trans* and *cis* taraxanthin. First dimension, adsorption chromatography with 5% acetone in 40–60° light petroleum. Second dimension, reversed phase chromatography with methanol across medicinal (liquid) paraffin.

*The Carotenol*

Pigment *t*1, produced by saponifying *d*1 and purified by chromatographing from 20% acetone in light petroleum, had the same spectral absorption maxima as those of the di-ester. This result conforms with Karrer and Jucker's[2] statement that "the spectral properties of xanthophyll and its esters are largely identical". Pigment *t*1 was strongly held by adsorbents, was only slightly soluble in light petroleum and was hypophasic to light petroleum over aqueous alcohols. The properties of *t*1 agree with those described by Strain[4] for taraxanthin.

*Mono-Ester*

Pigments *m*1 and also *t*1 had the same spectral maxima as those of the di-ester, and all three spectral absorption curves had the same shape. When saponified, *m*1 produced a

pigment indistinguishable from *t*1. Pigment *m*1 was approximately equally distributed between light petroleum and 90% acetonyldimethyl carbinol in water. After acetylation of *m*1 with pyridine and acetic anhydride (2:1) the ester produced ran close to *d*1 on a chromatogram. The above evidence suggests that pigment *m*1 is a mono-ester of taraxanthin.

*Di-Ester Content of Petals*

A few flowers were removed from each of several flower heads, weighed and extracted.[5] The predominant pigment *d*1, together with the much smaller quantity of *d*2, were separated from other pigments on alumina, and the extinction was read at 442 mμ. If the value for E (1%, 1 cm) for taraxanthin be taken as 2500* the average content of di-ester, in terms of free carotenol, was 350 μg/g. The contents of mono-ester and of free taraxanthin were lower.

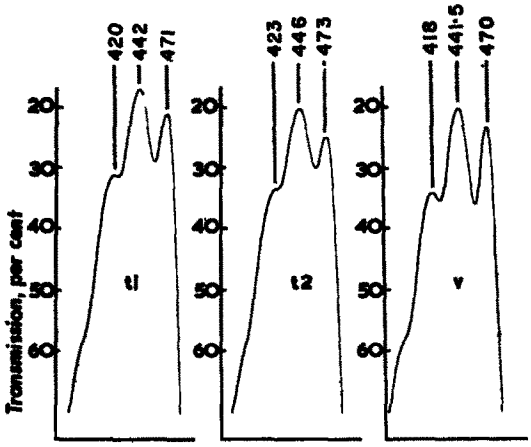


FIG. 2. SPECTRAL ABSORPTION CURVES COMPARED.

*t*1, Taraxanthin; *t*2, *cis*-taraxanthin; *v*, violaxanthin. Arbitrary concentrations. Solvent, ethanol. Wavelengths are shown at the absorption maxima. [Wavenumbers, in c/cm, are given in the text.] The figures are reproduced from direct tracings of the curves produced on a Unicam recording spectrophotometer.

*Isomerization*

Pigments *t*1 and *t*2 were separately eluted into acetone, transferred to light petroleum, placed in sunlight for an hour and rechromatographed side-by-side from 20% acetone in light petroleum. Both *t*1 and *t*2 appeared on *each* strip, accompanied by two other very faint bands. Repetition with *t*2 produced from *t*1, or *t*1 from *t*2, showed that again, each had produced some of the other. The process was repeated until the pigments were too weak for further manipulation. The interchanges occurred more slowly in the dark.

The obvious explanation for this phenomenon is *trans*→*cis*-isomerization.[7] Pigment *m*1 when treated similarly, produced some *m*2, and *d*1 some *d*2. The *cis* peak of *t*1 was much weaker than that of *t*2, suggesting that the former was the all-*trans* compound.[7] The spectral

* The value quoted by Goodwin,[6] based on Kuhn and Lederer,[1] is 2800. But values given by Goodwin for other carotenoids, including the closely-related violaxanthin, are mostly lower.

[5] V. H. BOOTH, *Analyst* **84**, 464 (1959).
[6] T. W. GOODWIN, in *Modern Methods of Plant Analysis*, Ed. K. PAECH and M. V. TRACEY, Vol. 3, p. 272, Springer, Berlin (1955).
[7] L. ZECHMEISTER, *Chem. Rev.* **34**, 267 (1944).

absorption maxima of *t*2 (eluted from chromatograms into ethanol in dim light and tested immediately) were 423, 446 and 473 mμ (Fig. 2). The 473 peak (wavenumber 21,150) of *t*2 was less prominent than that of *t*1, and the maximum at 423 (wavenumber 23,620) was only a shoulder. The red shift and the peak depression are analogous to those for *trans*→*cis*-trollixanthin described by Eugster and Karrer,[8] and the analogy supports the view that *t*1 is the *trans* isomer. Within each pigment pair the more strongly adsorbed member was usually the more abundant. This conforms with common experience that the all-*trans* carotenoid is the naturally-occurring isomer.

*Fatty Acid Composition*

The di-ester, whether chromatographically separated on alumina, on thin layer kieselgel, or two-dimensionally on paper, was always accompanied by an oil which interfered with the identification of the fatty acid(s) of the pigment. It was argued that partial hydrolysis followed by re-esterification might affect the pigment and the contaminating oil differently, so making their separation possible. The following procedure was used.

Di-ester *d*1 was eluted from chromatograms, partially saponified and rechromatographed, and the mono-ester *m*1 isolated and acetylated as described above. The principal product, now a di-ester, on chromatographing to separate it from colourless mono-esters, appeared near position *d*1. The pigment, apparently still accompanied by some oil, was interesterified.[9] The methyl esters of the fatty acids were microsublimed, leaving the pigment in the residue. They were separated with 10% polyethyleneglycoladipate on silicone-treated Celite in a Pye Argon Gas Chromatograph with a strontium ionization detector. Palmitic, oleic, palmitoleic and stearic acids were all present in the proportions of 4:2:2:1 and in excess of the theoretical amount for the ester.

*Differentiation of Taraxanthin from Violaxanthin*

The spectral absorption maxima of taraxanthin are close to those of violaxanthin,[4] and the two carotenoids run near one another chromatographically.[6] However, Strain[4] showed that acids produce different effects on the two pigments; and Eugster and Karrer[8] demonstrated small spectral differences.*

The differences have been further elaborated during the present investigation. The carotenols from a saponified extract of lucerne (*Medicago sativa*) leaf were appled to $ZnCO_3$-treated paper as single streaks side-by-side with streaks of *t*1. The chromatogram was developed in one dimension from 10% acetone in light petroleum. The less strongly adsorbed of the two principal pigments from the lucerne was assumed to be luteol. The other, which ran only slightly less far than *t*1, was assumed to be violaxanthin (*v*). The two pigments *t*1 and *v* were eluted separately into ethanol and their properties were compared.

1. Although spectral absorption maxima of *t*1 (420, 442 and 471 mμ,) and of *v* (418, 441·5 and 470 mμ) were almost identical the shapes of the curves were different (Fig. 2). These

* Eugster and Karrer's "taraxanthin" was prepared from *Impatiens noli-tangere:* the shape of its spectral absorption curve in benzene resembles that of violaxanthin (*v* of Fig. 2). Taraxanthin di-ester was prepared in this laboratory without saponification or the use of diethyl ether, from *T. Officinale* at room temperature in dimmest light practicable; the spectral absorption curve in benzene was recorded within 75 min of harvesting. The shape of this curve, with maxima at 429, 453 and 481 mμ, resembled that of *t*1 and differed from that given by Eugster and Karrer for their "taraxanthin". It seems that the latter pigment may not be identical with taraxanthin from *T. officinale.*

[8] C. H. Eugster and P. Karrer, *Helv. chim. Acta* **40**, 69 (1957).
[9] W. Stoffel, F. Chu and E. H. Ahrens, *Anal. Chem.* **31**, 307 (1959).

differences were confirmed with other preparations made over a number of seasons. The spectral shape for *v* is the same as that for Eugster and Karrer's violaxanthin.[8]

2. Portions of the two pigments were transferred to light petroleum, left in sunlight for an hour and rechromatographed. Pigment *t*1 produced two major bands as before, but *v* produced three, one being more strongly adsorbed than the starting material.

3. Portions of the two pigments were transferred to a diethylether–methanol mixture. The spectral absorption curves were recorded before and after the addition of hydrochloric acid.[10] The curve for *t*1 changed but little, whereas that of *v* changed considerably both in shape and intensity.

4. An extract of the flowers of pansy (*Viola tricolor*)—the original source of violaxanthin—was saponified. The pigments were chromatographed side-by-side with *t*1, and that most resembling *t*1 was eluted. Its spectrum and its isomerization products differed from those of *t*1.

*Leaves*

Extracts were prepared from leaves of *T. officinale*, and chromatographed. No esters of taraxanthin were observed.

## DISCUSSION

There can be no reasonable doubt that the pigment described herein is a di-ester of taraxanthin,* the latter having been so named following its isolation from *Taraxacum*. The di-ester was probably missed by earlier workers because they had saponified the pigments from dandelions before isolating taraxanthin.

The best-known carotenoid esters are helenien (luteol dipalmitate) and physalien (zeaxanthol dipalmitate).[2] The di-ester of taraxanthin resembles these in certain respects. The name taraxien, by analogy with these esters, is suggested for the di-ester of taraxanthin. By analogy with these carotenoids, the di-ester presumably contains palmitate; however much it was purified it always yielded an excess of palmitate on saponification.

This is the first reported occurrence of a di-ester of taraxanthin, and although di-esters of other carotenoids have been described, and mono-esters have been produced chemically,[2] this is also believed to be the first report of the presence of a carotenoid mono-ester in plants. If taraxanthin is unsymmetrical, as is α-carotene for example, two mono-esters could exist. No indication of two compounds was seen on the chromatograms, but of course the adsorption affinities might be closely similar.

## METHODS

For quantitative tests several whole flower heads of dandelion were weighed, extracted, and the principal pigment determined as in the method used for carotene,[12,5] except that the desired pigment was in the fraction eluted from the chromatographic column of alumina with 4% acetone in light petroleum, and that the extinction was measured at 442 mμ.

* Zechmeister and Tuzson's[11] "taraxanthin" differed in that its isomerization products were more strongly adsorbed than the all-*trans* compound. However, these authors gave no indication of the origin or method of preparation of their material; nor did they give its whole spectral curve, which might have helped to confirm its identity. Moreover, it may be significant that Zechmeister, in his classical review,[7] did not mention his taraxanthin. [See also footnote, p. 232.]

[10] A. L. Curl and G. F. Bailey, *J. Agric. Food Chem.* **9**, 403 (1961).
[11] L. Zechmeister and P. Tuzson, *Ber. dtsch. chem. Ges.* **72**, 1340 (1939).
[12] V. H. Booth, *Carotene, its Determination in Biological Materials*, Heffer, Cambridge (1957).

For qualitative experiments, extracts were made by steeping the flower heads in light petroleum in the dark. The extracts were concentrated, then chromatographed on paper in various ways. Whatman No. 4 filter papers (22 × 32 cm), were treated with $ZnCO_3$.[13] Extract was applied to the paper with a Trenner-type pipette[14] and chromatograms developed in the first dimension by the ascending method using various concentrations of acetone in light petroleum. For the second dimension, reversed phase chromatograms were developed by the ascending method with methanol across medicinal (liquid) paraffin.[14] For the comparison of pure pigments side-by-side on one paper, only the first dimension chromatography was used.

For larger quantities of pigment, three Whatman No. 31 $ZnCO_3$-treated papers were rolled into loose spirals.[14] Each was dipped in turn into pigment extract about 2 mm deep in a large beaker, then removed, inverted and allowed to dry briefly and the dippings repeated. Acetone was then added to the beaker to drive pigments from the bottom of the paper. Chromatograms were then developed with, for example, 1% acetone in light petroleum. The required pigments were eluted with acetone or alcohols.

Pigments were saponified cold by shaking for ½ min in a mixture of light petroleum 40–60°, propanol, methanol and saturated aqueous potassium hydroxide (approx. 2:1:1:2 v/v[12]). After a further 20 min when complete hydrolysis was required, or ½ min for partial hydrolysis, the lower layer was discarded and the upper layer was washed with water.[12]

Spectra were recorded on a Unicam SP700. Checks were made immediately before and immediately after every run, and wavenumber corrections were made where necessary. All results are the means of at least duplicate runs.

*Acknowledgements*—I thank Mr. M. P. Bradford for skilled technical assistance. I am grateful to Mr. P. W. Wilson for the analysis of fatty acids by gas–liquid chromatography and interpretation of the results.

[13] J. GREEN, S. MARCINKIEWICZ and P. R. WATT, *J. Sci. Fd Agric.* **6**, 274 (1955).
[14] V. H. BOOTH, *Analyst* **88**, 627 (1963).